ELECTROPHORETIC SKIN STUDIES

I. Reaction to Common Grasses

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INTRODUCTION

By intradermal and scratch testing it has been shown that persons with clinical allergy due to grass sensitivity are usually found skin sensitive to extracts of timothy grass and also to the extracts of many of the common grasses. It is assumed that a protein-like element contained in these grass antigens produces the skin reactions and is the causative factor in the production of the allergic symptoms. Many allergists consider the extracts of timothy grass as the common sensitizing factor in all the grass cases and treat these patients by hyposensitizing them with gradually increasing doses of these extracts. By this simplified method of treatment, as good results are obtained as those treated with extracts of the "mixed grasses."

Abramson (1) has shown that active allergens of timothy grasses, giant and dwarf ragweed may be transported readily into the skin of hypersensitive persons by electrophoresis. These findings were later confirmed by Dutton (2) who also investigated other substances.

With this in mind, patients with known skin sensitivity to extracts of timothy were tested iontophoretically with extracts of different grasses to determine whether or not the skin reactive constituents of these extracts could be readily transported into the skin by this method; and if so, could one also demonstrate the presence of a parallelism between the reactions of the common grasses by intradermal testing and by electrophoresis?

Five different patients found skin sensitive to extracts of timothy grass by intradermal testing were studied iontophoretically with the extracts of various grasses, i.e., orchard, red top, sweet vernal, and June grasses. Four of these patients have had allergic symptoms during the grass season, two of whom have dermographic skins. The remaining case with dermographism, has never suffered with grass pollen fever (potential).

METHODS

Undialyzed extracts (1) of timothy, orchard, red top, sweet vernal and June grasses were used, each containing 0.5 milligrams of total nitrogen per cubic centimeter. The pH of the solutions were adjusted to 7.4 and the solutions were preserved with glycerine at the suggestion of Abramson (3) who has been using glycerine solutions in unpublished experiments on the treatment of hay-fever electrophoretically. Scratch tests were made with varying strengths of

TABLE 1

CASE NUMBER	SUMMARY OF HISTORY	GRASSES BY IONTOPHORESIS	REACTIONS	SCRATCH	REACTION
				mgm. N/cc.	
1	♀, age 23. Complaining of H.F. symptoms spring and summer. Intradermal tests: Rag. Class CD; Tim. Class C; Dermographic skin	Timothy	Confluent large wheals; itching	0.1	sl. +
		Orchard Grass	Large confluent wheals; itching	0.1	str. +
		Sweet Vernal	Large confluent wheal; itching	0.1	str. +
		June Grass	Minute discrete blebs; sl. itching	0.1	str. +
		Red Top	Large confluent wheal; itching	0.1	str. +
2	♂, age 18. Complaining of H.F. symptoms August 15 to October 1. Intradermal tests: Rag. Class BC-C, Tim. Class C-BC (Potential Tim. case), Dermographic skin	Timothy	Confluent large wheals; itching	0.1	str. +
		Orchard Grass	Many minute wheals; itching	0.1	str. +
		Sweet Vernal	Many minute wheals; itching	0.1	sl. +
		June Grass	Few discrete blebs; itching	0.1	str. +
		Red Top	Large confluent wheals; itching	0.1	str. +
3	♂, age 17. H.F. symptoms latter part of June to October 1. Intradermal tests: Rag. Class C-BC, Tim. Class C-BC, Dermographic skin	Timothy	Large confluent wheals; itching	0.5	str. +
		Orchard Grass	Discrete minute blebs; no itching	0.5	str. +
		Sweet Vernal	Few discrete blebs; no itching	0.5	mod. +
		June Grass	Large discrete wheals; itching	0.5	str. +
		Red Top	Large confluent wheals; itching	0.5	str. +
4	♂, age 23. H.F. symptoms middle of May to end of June. Intradermal tests: Tim. Class CD-C, Trees Class C	Timothy	Many discrete blebs; itching	0.5	str. +
		Orchard Grass	Many discrete wheals; itching	0.5	str. +
		Sweet Vernal	Many minute blebs; no itching	0.5	mod. +
		June Grass	Large confluent wheal; itching	0.5	str. +
		Red Top	Large confluent wheal; itching	0.5	str. +
5	♀, age 46. Perennial asthma. H.F. symptoms middle of May to end of June. Intradermal tests; Rag. Class CD, Tim. Class B	Timothy	Large confluent wheal; itching	0.1	str. +
		Orchard Grass	Large confluent wheal; itching	0.1	str. +
		Sweet Vernal	Large confluent wheal; no itching	0.1	str. +
		June Grass	Large confluent wheal; itching	0.1	str. +
		Red Top	Large confluent wheal; itching	0.1	str. +

these glycerinated solutions (0.1 mg. total nitrogen to 0.5 mg. total nitrogen per c.c.) in order to obtain a general idea as to the sensitivity of the patient to these extracts before applying them electrophoretically. In this way the possibility of systemic reactions were diminished.

A simple galvanic current was employed. Each extract was applied separately at the positive pole by saturating Canton flannel, 1 centimeter square, with it; employing from one-half to two millamperes of current over a period of two minutes.

The results of these experiments are outlined in table 1. In this table a comparison of the reactions obtained from this group of patients with iontophoresis and the scratch method of testing are shown. In interpreting the degree of reactions with the scratch method the usual criteria were used. With iontophoresis the degree of skin reactions were interpreted as follows:

Negative: Slight erythema; no papules.
Slight positive: Erythema with few discrete papules.
Moderate positive: Large area of erythema with many discrete papules.
Strong positive: Confluent wheals, with large area of erythema and itching.

It is shown in table 1 that the electrophoretic method not alone introduces timothy into the skin but also biologically active constituents from the other extracts. Abramson, Moore, and Gettner (4) have shown that the molecular weight of Trifidin, the major colorless component of gaint ragweed extract, is about 5000. This is below the molecular range of proteins like egg albumin which is closer to 40,000. The fact that these substances are introduced electrophoretically with such facility is in all likelihood connected with the small size of the molecule as shown by Abramson, Moore, and Gettner, although this has not definitely been shown. Abramson has reported (personal communications) that he has separated a major colorless component of timothy (with Moore and Gettner) and is investigating the molecular weight of the major constituent of the grasses in table 1. These workers are attempting to correlate the diffusion constant and the molecular weight with the ease with which these materials may be forced into the skin by electrophoresis and with which they cause clinical manifestations.

Note also, in table 1 that there was a certain degree of parallelism between the intensity of the reaction found by electrophoresis and the intensity of the reaction observed by the scratch test. It should be realized, however, that the intensity of the reaction by electrophoresis may be readily changed by changing the concentration, the time, and the current density.

SUMMARY

1. Biologically active constituents of orchard, red top, sweet vernal, and June grasses can be transported readily into the skin by electrophoresis in persons allergic to timothy grass.

2. This can be demonstrated in persons with present or past clinical allergy.

3. The skin reactions produced by iontophoresis, in general, are parallel to

the skin reactions as demonstrated by the usual skin tests (scratch and intradermal).

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REFERENCES

(1) ABRAMSON, H. A.: Science, **87:** 299, 1938.
ABRAMSON, H. A.: J. Allergy, **12:** 169, 1941.
(2) DUTTON: J. Allergy, **12.**
(3) ABRAMSON, H. A.: Personal communications; also Annual Rep. Biol. Lab., Cold Spring Harbor, 1940, p. 19.
(4) ABRAMSON, MOORE, AND GETTNER: Proc. Soc. Exper. Biol. and Med., **44:** 311, 1940. Ibid., **46:** 153, 1941.